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Polymer implants for drug delivery in the brain

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The delivery of drugs to the brain represents a complex and important area. In this paper we review our research in four areas: (1) development of experimental techniques to "paint" controlled release polymers on brain microvessels to release proteins, (2) controlled release of dopamine from polymer matrix systems for the potential treatment of Parkinson's disease, (3) controlled release of bethanechol from biodegradable polymeric microspheres for potential treatment of Alzheimer's disease, (4) controlled release of nitrosoureas from biodegradable polymer discs for potential treatment of brain cancer.

Keywords: Polymer implant; Horseradish peroxidase; Biodegradable polymeric microspheres; Polymer matrix systems; Biodegradable polymer discs

Introduction

A significant difficulty in the treatment of many neurological disorders is the inability to deliver drugs to the brain, particularly within discrete regions, and at a controlled or constant rate. The primary cause for this problem is the blood-brain barrier which precludes the entry of many substances. Even if such a substance could bypass this barrier, it would most likely be distributed throughout the brain in a relatively undefined manner. A number of methods have been attempted to address these problems, such as osmotic disruption, infusion pumps and the implantation of adrenal or fetal neural tissue (for review, see 1). Each of these methods has significant deficiencies, including toxic side effects, size, safety, reliability and cost.

We have attempted to overcome these diffi-

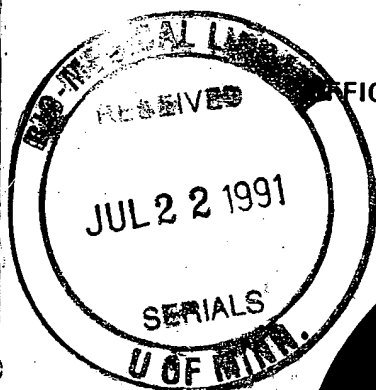
culties by developing biocompatible polymeric systems which permit the controlled and localized release of neuroactive substances directly into the brain. We review here four studies in which we have participated to examine controlled release to the brain. These are (1) the release of enzymes to brain blood vessels to provide histochemical localization, (2) dopamine release systems for potential treatment of Parkinson's disease, (3) bethanechol release systems for potential treatment of Alzheimer's disease and (4) BCNU [*N,N* bis(2-chloroethyl)-*N*-nitrosourea] (also known as carmustine) release for the potential treatment of brain cancer.

Controlled release of horseradish peroxidase from polymers: a method to improve histochemical localization and sensitivity

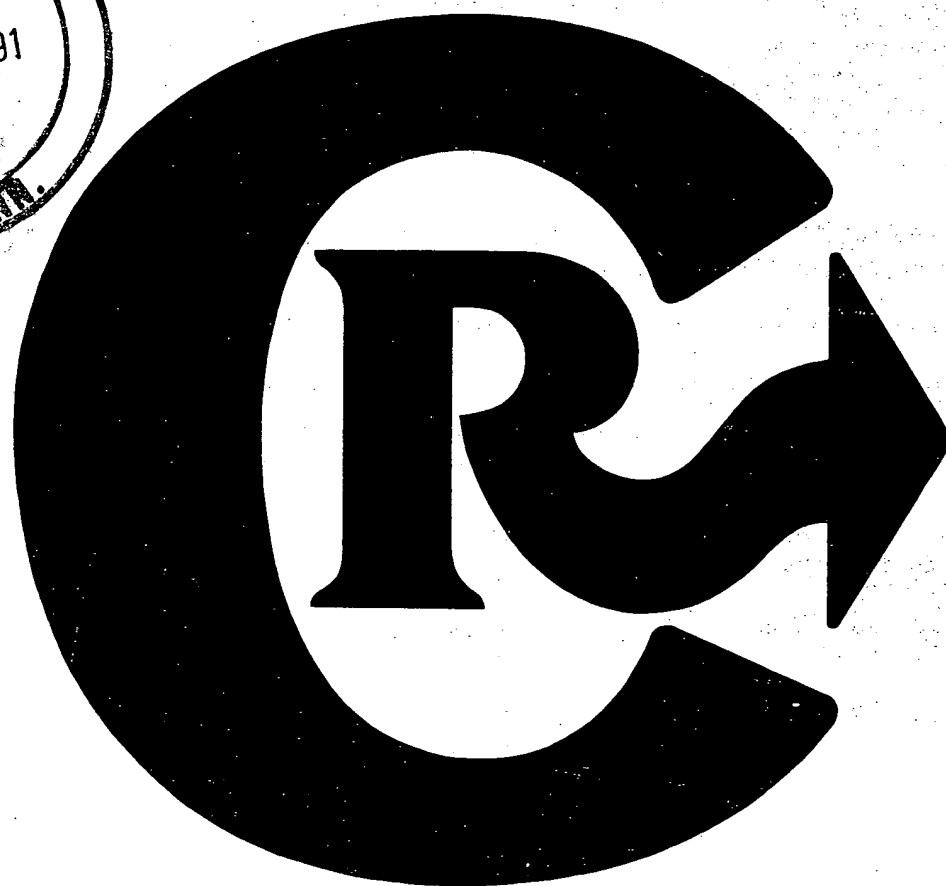
This study provided, to our knowledge, the first example of controlled release polymer systems in the brain [2]. The protein, horseradish peroxidase (HRP), is an important tool for histochem-

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ically defining neuronal connections within the peripheral and central nervous system. Most often, HRP is administered to discrete brain regions as an aqueous solution, either by stereotaxic injections or by microiontophoresis. Outside the central nervous system HRP is injected directly into, or placed on a target organ or applied to the proximal end of a severed nerve. One difficulty which limits the utility of HRP histochemistry is the diffusion of enzyme away from the site of injection. This marker enzyme is often taken up by adjacent nerve endings and transported retrogradely to yield false positive results. This is especially problematic when HRP is used to trace pathways between specific areas of the brain, such as the meninges, ventricular surfaces, cranial nerves or cerebral blood vessels. This first study [2] describes a new technique for localizing HRP by incorporating it into a controlled release polymer which can be applied simply by "painting" it onto cerebral blood vessels. Both in vitro and in vivo tests demonstrated the effectiveness of this technique.

In this case, a casting solution of poly(vinyl alcohol) was first prepared as described in [3]. In addition, a solution of pluronic F-127, a polyol, was prepared by slowly dissolving 20 g of solid polymer into 100 ml of cold water in a beaker while mixing with a magnetic stirrer.

Initial studies examined the rate at which HRP was released from poly(vinyl alcohol) in vitro at 25°C. Approximately 69% of the incorporated horseradish peroxidase was released by the poly(vinyl alcohol) within 24 h. Release was continuous and the enzyme activity was retained after HRP was liberated from the polymer [2].

Experiments were next conducted to examine the extent to which HRP diffuses away from the blood vessel after it is released from the polymer. Since meningeal tissue (the tissue covering the brain underneath the skull) contains exogenous peroxidase activity, we examined the lateral migration of HRP by fluorescence microscopy using HRP conjugated to fluorescein isothiocyanate. Poly(vinyl alcohol) solution containing HRP and/or fluorescein-conjugated HRP was placed onto a meningeal blood vessel; unimpreg-

nated polymer solutions (either PVA or pluronics) were placed around it to act as barriers. This was done in several steps: (1) a barrier was constructed along the lateral edges of the vessel as well as proximal and distal to the site of interest using PVA or pluronics. Pluronics was used because of its unusual property of solidifying upon contact with temperatures above 15°C. This polymer solution was maintained at 40°C until it was applied with a chilled pasteur pipette, (2) poly(vinyl alcohol) containing HRP was then applied to the surface of this meningeal artery with a microspatula, (3) unimpregnated PVA or pluronics was then used to cover the coated vessel so as to limit release solely to the interface between the unimpregnated polymer and the blood vessel, (4) parafilm was then placed over the polymer to prevent spread to the adjacent dura (the dura is the top meningeal layer), and the skin flap closed to protect the exposed brain surface. At 6 and 12 h after this application, the skin flap was opened, and the diffusion of marker enzyme was assessed by ultraviolet light. The intensity of fluorescence diminished markedly at 6 and 12 h, but remained confined to the surface of the blood vessel. Tissue sections viewed by fluorescent microscopy confirmed that nearly all of the HRP remained within 10 mm lateral to the vessel and 5 mm beneath the adjacent cerebral cortex [2].

The ability of HRP released from polymer to be taken up and transported by local nerve terminals was next examined. Approximately 3 mg of horseradish peroxidase was mixed with 3.0 g of poly(vinyl alcohol) and applied as described above to the proximal 2 cm segment of the middle cerebral artery in the cat (for experimental details concerning animal preparations, see [2]). After 72 h, the animals were perfused with at least 600 ml of 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Thirty min later, brain tissue from the superior cervical ganglia and trigeminal ganglia was removed along with a representative section of the middle cerebral artery and underlying cortex. This tissue was post-fixed in 2.5% glutaraldehyde solution for 2 h and prepared for peroxidase staining. Histological

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sections were examined both by bright field as well as dark field microscopy. Cell bodies of the reaction product were identified within the ipsilateral superior cervical ganglia and trigeminal ganglia [2].

These studies demonstrate the advantages of using controlled release polymers as a means of localizing and delivering HRP *in vivo* and *in vitro*. When this enzyme is administered via the polymer, spread beyond the designated tissue is reduced to less than 1 cm lateral to the cerebral vessel of interest and contact with local structures is prolonged. The spread was also reduced by coating the external surface of the impregnated poly(vinyl alcohol) with pure polymer. By doing so, the release of HRP was restricted towards the surface of the blood vessel.

The simplicity with which these poly(vinyl alcohol) solutions can be "painted" onto blood vessels opens new possibilities for drug localization and for neuroanatomical studies defining nerve pathways from superficial structures. One example of where we used this approach involved defining possible pathways for vascular headaches [4].

Dopamine release systems

In this case, dopamine was used as a model drug and ethylene-vinyl acetate copolymer as a model polymer to examine matrix systems as a way to deliver drugs to the brain. Fig. 1 demonstrates the cumulative *in vitro* release of dopamine from a variety of conventional polymer matrix configurations (e.g. discs). Cumulative release was directly proportional to the square root of time, suggesting that diffusion of the encapsulated dopamine from the polymer was the rate-limiting step in the release process. However, we have shown that matrix systems that are coated with impermeable coatings with permeable apertures can provide constant release [5]. Thus, when a single aperture was introduced into a matrix with an impermeable coating, release was linear with time. Release rates of 0.06, 0.17 and 0.30 mg/day were obtained from matrices of 30, 40 and 50% loading respectively [6].

The systems with the single apertures were then

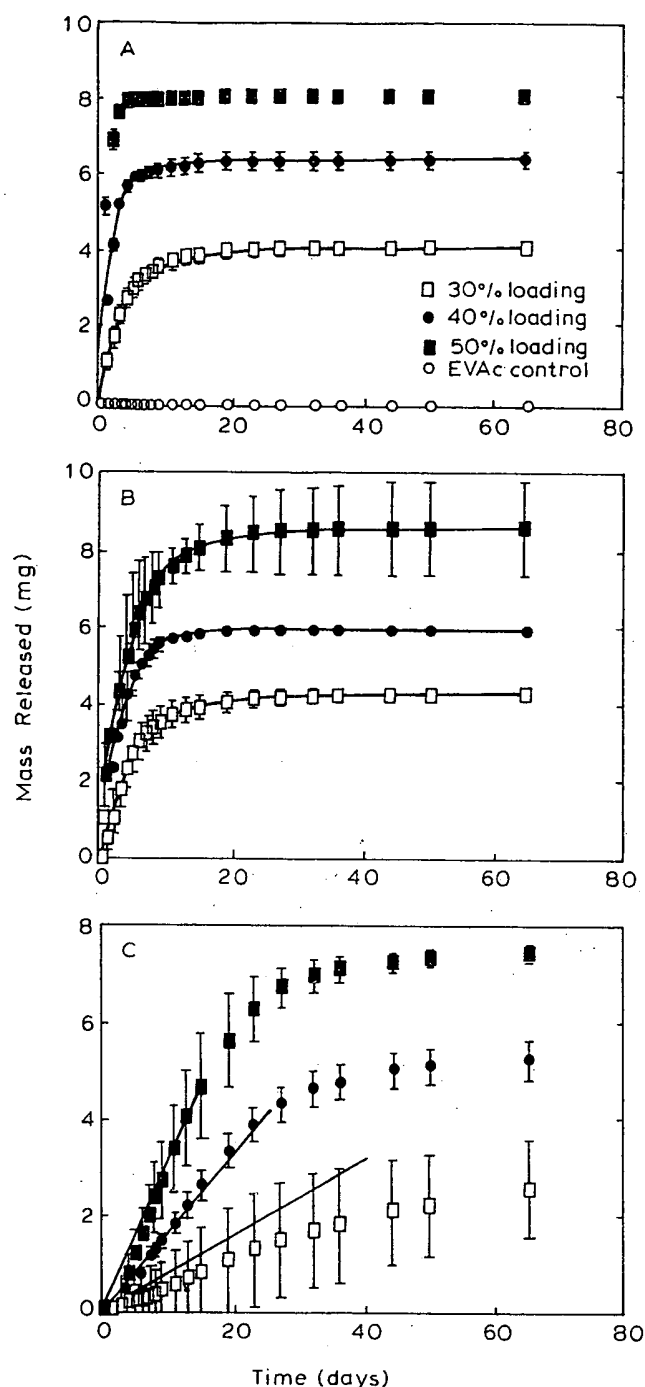


Fig. 1. Release of dopamine from dopamine/ethylene-vinyl acetate copolymer matrices. Each experimental point represents the mean (\pm standard deviation) cumulative mass of dopamine released for four sample matrices. Each dopamine/polymer matrix initially contained 0% (empty circle), 30% (empty square), 40% (filled circle), or 50% (filled square) by weight dopamine. The cumulative mass of dopamine released is shown for matrices with different geometries: (A) a simple disc, (B) a disc with one impermeable face, and (C) a disc with a completely impermeable coating except for a single cavity in one face. Solid lines in the bottom panel demonstrate the linear release predicted by a model of diffusion of dopamine through the prescribed geometry. (Taken from Reference 6).

implanted into the brains of rats [7]. Dopamine concentrations in the extracellular fluid of a brain structure known as the striatum in normal rats that received a control implant and normal rats without any implant were stable over the course of these experiments: 29 ± 5 nM and 22 ± 5 nM, respectively. In contrast, extracellular concentrations of dopamine reached unprecedented elevations of as much as $7.2 \mu\text{M}$ at days 10 through 65 after implantation of the dopamine-polymer device in the ipsilateral corpus striatum of experimental rats. Stable levels were reached at 20 days after implantation and were maintained throughout the length of the experiment (day 65) (Fig. 2). The stability of dopamine release was further demonstrated with a prolonged dialysis experiment on 2 rats which was performed at 45 days after polymer implantation; stable release of dopamine over a period of 5 h was observed. Levels of dopamine (26 ± 4 nM) in the contralateral, left side in rats with dopamine-polymer implants showed no difference from levels in the right striatum of rats with the control polymer implant or normal rats.

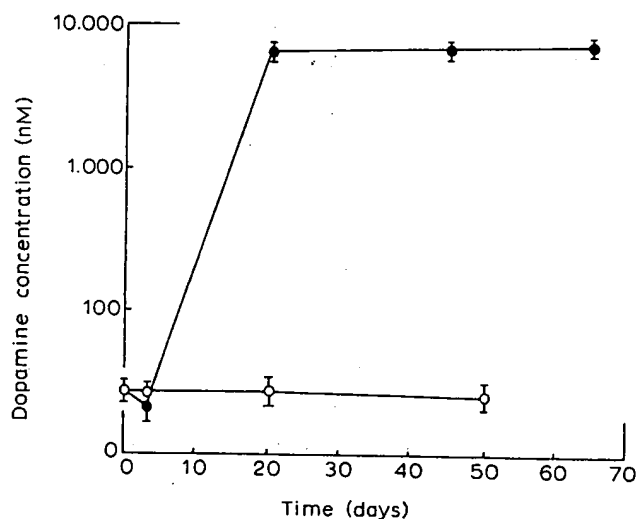


Fig. 2. Time course of in vivo dopamine release. Concentrations of dopamine in extracellular fluid were estimated using intrastriatal microdialysis. Each value represents the mean (\pm SEM) of a minimum of four measurements. A total of 12 rats (dark circles) received a dopamine-polymer device (30% loading, fully coated except for one cavity), and 4 rats (open circles) received a control polymer device. Animals were randomly selected for dialysis at the indicated time points. (Taken from Reference 7).

As judged histologically, the dopamine-polymer devices were located throughout the striatum; within $500 \mu\text{m}$ of the implant-striatal interface, striatal morphology was normal. All rats survived this study [7].

Bethanechol release from degradable microspheres

Alzheimer's disease is the most common cause of severe dementia in the United States, affecting over one million people. According to the "cholinergic hypothesis" of Alzheimer's disease, characteristic clinical deficits in memory and learning are a result of inadequate cortical levels of acetylcholine. This hypothesis has provided the rationale for a variety of treatment methods [8,9].

Pharmacological treatment using precursors of acetylcholine synthesis, cholinergic agonists and acetylcholinesterase (AChE) inhibitors has largely been disappointing in patients with Alzheimer's disease. The poor functional outcomes and high incidence of adverse side effects may have resulted in part from a lack of regional specificity of these drugs within the brain, since conventional drug delivery systems are incapable of continuously delivering therapeutic agents to selective subregions of the brain. To examine the ability of controlled release polymers to address this problem, a 50:50 copolymer of poly(bis(*p*-carboxy-phenoxy)propane) anhydride and sebacic acid (PCPP-SA) in the form of 3 to $5 \mu\text{m}$ microspheres was used to provide continuous release of bethanechol over 16 to 20 weeks.

The microcapsules were implanted in the denervated hippocampus (a part of the brain known to be important in memory) in rats to provide selective local administration of the drug [10].

AChE histochemical findings revealed widespread loss of hippocampal staining optical density in the animal model employed in this study – fimbria-fornix lesioned rats – compared to unoperated control rats. Following fimbria-fornix lesioning, there was a consistent 50% to 70% reduction [10].

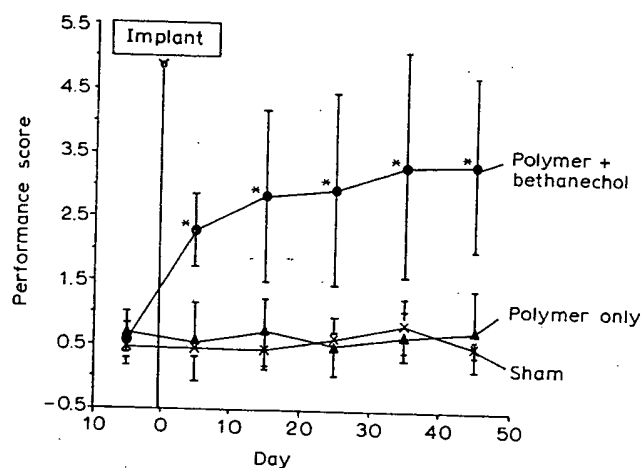


Fig. 3. Group maze performance scores in the three treatment groups: sham (five saline-treated rats), blank polymer (five rats), and bethanechol-impregnated polymer (10 rats). The vertical axis denotes the mean corrected group performance score over each 10-day epoch \pm standard deviation; the horizontal axis denotes the number of days before and after hippocampal implantations. The performance of the bethanechol-impregnated polymer group shows significant improvement compared to baseline at all postimplant time intervals. Asterisks indicate statistically significant changes ($P < 0.05$, Student's *t*-test). (Taken from Reference 10).

All rats tolerated the hippocampal implants well. No difference in general activity level was noted among the various treatment groups. The animals implanted with polymer-bethanechol (microspheres) entered fewer than eight arms in an eight arm maze test in 7.3% of all trials, compared with 8.4% in the blank polymer-treated groups and 7.0% in the saline-treated group. In the succeeding weeks, the control rats treated with saline and blank polymer continued to perform poorly, showing no improvement in performance scores above baseline at any of the time intervals tested. As a group, the rats treated with bethanechol-impregnated microspheres improved significantly following implantation ($P < 0.05$). Individual performances, however, varied within this improved treatment group; some rats ran the maze without mistakes while others showed only modest improvement. The time course of improvement was rapid, reaching statistical significance during the first 10-day epoch. Beneficial treatment effects persisted for the entire 40 days of the experiment [10] (Fig. 3). Thus, this study illustrates a new method of in-

tracerebral regional neurotransmitter delivery that can be utilized to reverse lesion-induced spatial memory deficits in rats with bilateral fimbria-fornix lesions.

BCNU (carmustine) in degradable polymers to treat brain cancer

Severe forms of brain cancer, termed glioblastoma or astrocytoma grade 3 on 4, are one of the most deadly diseases known to man. The average life span of patients afflicted with such a disease is 4 weeks if left untreated and less than a year regardless of the treatment method. The most common drug used to treat brain cancer, BCNU, is normally given intravenously. However, it has a half-life of 12 min and therapeutically effective systemic levels cause damage to numerous body organs. Therefore local controlled release was proposed to lengthen the lifetime of BCNU and to localize it to the brain.

BCNU has been incorporated into PCPP:SA 20:80 in two different ways. These involve either (1) triturating dry powdered BCNU with similarly treated polymer and pressing weighed aliquots of the mixture in a Carver press, or (2) co-dissolving polymer and BCNU in methylene chloride, evaporating the solvent, and pressing the resulting material as in method 1. The former method produces a solid mixture of BCNU and polymer, while the latter method produces a solid solution of BCNU in the polymer. These are referred to here as the trituration or solution methods.

In *in vitro* studies, BCNU at 2–3% loading was completely released from the wafers prepared by the trituration method within the first 72 h, whereas it took nearly twice as long for the BCNU to be released from wafers produced by the solution method. Essentially the same release pattern was observed for wafers loaded with 10% BCNU by weight [11].

The differences in rates of release of BCNU from wafers produced by the trituration or solution methods are also seen *in vitro*. Wafers of PCPP:SA 20:80 were prepared by either the solution or trituration methods, as described above and were implanted into the brains of rabbits.

The animals were sacrificed at various times after implantation and the brains were removed, fixed and processed for quantitative autoradiography. The rate of release of BCNU from wafers prepared by the solution method was slower than that from wafers prepared by the trituration method, in agreement with the *in vitro* results. At the longest time measured, 21 days after implantation, these animals had significant levels of BCNU in an area of brain almost twice as large as those receiving the wafers produced by the trituration method [12].

The use of this polymeric delivery system for BCNU greatly increases the time over which the brains of these animals are exposed to significant BCNU concentrations. The brains of animals which received a single injection of the same amount of BCNU contained in the wafers were almost free of BCNU three days after injection. Since it has been shown conclusively both *in vitro* and *in vivo* that the key determinant of the ability of a drug to kill cancer cells depends on the product of the drug's concentration and the time over which the drug and cancer cells are in contact [13,14], it seems reasonable to conclude that the greatly increased time over which BCNU is delivered to the brain using PCPP:SA 20:80 should increase the efficacy of BCNU.

From these same autoradiography studies, it is also possible to determine the local brain concentrations of BCNU which can be achieved using this polymer delivery system. The brain adjacent to the surface of these wafers is exposed to concentrations of BCNU of approximately 6.5 mM at three days following implantation. Even as far as 10 mm from this surface, the local concentrations of BCNU are approximately 200 μ M (Fig. 4). This concentration range is certainly much higher than the brain concentrations achieved through a single intravenous administration of BCNU, which is the standard treatment for this disease.

Given these initial results and the autoradiography studies, four additional sets of preclinical studies were conducted: (1) safety studies of high doses (up to 800 times human dose) of polymer in rats [15], (2) safety studies of polymers in

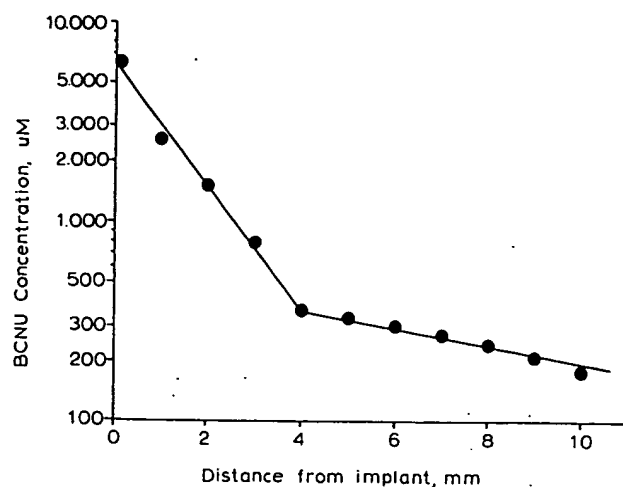


Fig. 4. The movement of [3 H] BCNU-associated radioactivity through the rabbit brain. Radioactivity resulting from [3 H] BCNU was measured at various distances from the implantation site following implantation of polyanhydride discs into the brains of rabbits. (Taken from "Polyanhydrides as Drug Delivery Systems", in R. Langer and M. Chasin (Eds.), *Biodegradable Polymers as Drug Delivery Systems*, Marcel Dekker Inc., N.Y., (1990) pp. 43-70.

rabbit brains [16], (3) safety studies of polymers in monkey brains, (4) efficacy studies of polymer-BCNU in rats with 9L glioma [17]. All of these studies showed the polymeric BCNU delivery device to be safe and effective in animals.

PCPP:SA 20:80 discs with BCNU incorporated into it, was next studied in man for the treatment of glioblastoma multiforme, a universally fatal form of brain cancer [17]. In these studies, patients undergoing reoperation for the removal of the bulk of the tumor had the surgical cavity lined with polymer discs containing BCNU. Following surgery, the BCNU is then released directly into adjoining tissues that may contain cancer cells not removed during surgery. The safety of this material implanted into these patients has been demonstrated in a phase I/II clinical trial of 21 patients in 5 U.S. hospitals. No systemic side effects of doses of BCNU which would produce marked effects on the hemopoietic system when injected intravenously have been observed [18]. A phase 3 clinical trial examining this system in 32 U.S. hospitals is currently under way.

In summary, the above studies show that controlled release polymers may be useful in study-

ing and potentially treating a variety of brain disorders. As new agents (e.g., nerve-growth factors) which have new types of pharmacological activity are uncovered, such polymer systems may play a role in the testing and perhaps eventual treatment of new therapeutic modalities in the brain.

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